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Initial Toxicological Assessment of Ambush, Bolero, Bux, Dursban, Fentrifanil, Larvin, and Pydrin: Static Acute Toxicity Tests with Selected Estuarine Algae, Invertebrates, and Fish

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16. ABSTRACT

Selected static toxicity tests were conducted with Ambush®, Bolero®, Bux®, Dursban®, Fentrifanil®, Larvin®, and Pydrin® to determine the sensitivity of species representing four major phyla. Algal bioassays were conducted with marine algae to determine the concentration of pesticide that would inhibit population growth by 50% in 96 h. Static toxicity tests with mollusk larvae estimated the concentration of pesticide that would cause 50% of the exposed larvae to develop abnormally in 48 h. Static acute lethality tests with crustaceans and fish determined the concentration of pesticide that is lethal to 50% of the test organisms during a 96-h exposure.

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INITIAL TOXICOLOGICAL ASSESSMENT OF AMBUSH, BOLERO, BUX, DURSBAN, FENTRIFANIL, LARVIN, AND PYDRIN: STATIC ACUTE TOXICITY TESTS WITH SELECTED ESTUARINE ALGAE, INVERTEBRATES, AND FISH

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INTRODUCTION

The sensitivity of estuarine organisms to seven pesticides granted experimental use permits or awaiting registration was evaluated in response to a request of the Office of Pesticide Programs, Ecological Effects Branch, Hazard Evaluation Division, U.S. Environmental Protection Agency (EPA). Acute static toxicity tests were conducted with six insecticides (Ambush[R], Bux[R], Dursban[R], Fentrifanil[R], Larvin[R], and Pydrin[R]), and one herbicide (Bolero[R]) by the EPA Environmental Research Laboratory, Experimental Environments Branch, at Gulf Breeze, Florida (ERL,GB). Sources, purities, target pests, applications, and registration status are summarized in Appendix A.

This report is the first of a series and includes the biological effects "screening" data generated in 96-h EC50 (concentration of pesticide that would inhibit growth by 50%, as compared to control growth in 96 h) bioassays with marine algae, 48-h EC50 (concentration of pesticide that would cause 50% of the exposed larvae to develop abnormally in 48 h) tests with estuarine mollusk larvae, and static acute 96-h LC50 (concentration of pesticide that is lethal to 50% of the test organisms during a 96-h exposure) toxicity tests with estuarine crustaceans and fish.

MATERIALS AND METHODS

Algal Bioassays

Algal Species Tested and Procedures

One to four species of algae were used in tests with Ambush, Bolero, Dursban, Larvin, and Pydrin. Tests with all five pesticides were conducted with Skeletonema costatum. In additional tests, Isochrysis galbana and Thalassiosira pseudonana were exposed to Dursban and Pydrin. Nitzschia angularum was also tested with Pydrin. All were obtained from the Culture Collection of Algae, University of Texas, Austin, TX, except S. costatum which was obtained from the University of Rhode Island, Kingston, RI.

Media for stock algal cultures and for tests pesticides were prepared by dissolving a commercial artificial sea salt formulation (Rila Products, Teaneck, NJ*) in deionized water to the concentration of 30 parts per thousand. Before the solution was taken to final volume, trace metals, vitamins, and minor salts were added as nutrients (Apprendix B). Medium was prepared on the day before a test was to begin to allow the pH to stablize at 8.1.

Algal stocks were maintained in 150 mℓ of culture medium with EDTA in 500 mℓ Erlenmeyer flasks under the following conditions: light intensity, 150 $\mu E/m^2/sec$(PAR, 400-700 nm waveband) from overhead "cool white"

[R] Registered trade marks, generic names and manufacturers are listed in Appendix A. Mention of commercial products or trade names does not constitute endorsement by the U.S. Environmental Protection Agency.

fluorescent tubes; lighting cycle, 14 h light followed by 10 h darkness; temperature, 20.0 ± 0.5°C; transfer to new stock medium every 10 days. Stock cultures were not shaken.

Pyrex^{R} (Corning Glass Works, Corning, NY) glassware was used and was prepared as follows: (1) soaked in detergent overnight, (2) brushed lightly, (3) rinsed thoroughly in tapwater, (4) rinsed three times in pesticide-grade acetone, (5) soaked overnight in 1% HCl, (6) rinsed thoroughly with tapwater, (7) rinsed five times with deionized water, (8) dried in an oven, and (9) stored in a relatively dust-free container or individually sealed with aluminium foil. All glassware and media were sterilized by autoclaving at 121°C and 1.4 kg/cm^2 for 20 min.

Bioassay Procedure

One week prior to the tests approximately 5 mℓ of an algal culture in logarithmic growth phase was added to 150 mℓ of culture medium that contained ethylenediaminetetracetic acid (EDTA), thus ensuring healthy culture in the logarithmic growth phase. On the day of the test, cultures were diluted with growth media to yield 2000 cells/mm^3 for S. costatum and 1000 cells/mm^3 for all other species; 1 mℓ of this cell suspension was added to each algal culture tube.

In tests with pesticides, 25 mℓ of culture medium without EDTA were pipetted into optically matched test tubes (25X200mm) which then were capped with stainless steel microbiological closures and autoclaved. On the second day, a known quality of pesticide in 0.1 mℓ of acetone was added to exposure tubes, and 0.1 mℓ of nanograde acetone was added to control tubes. Then 1 mℓ of algal suspension was added aseptically to each tube. Treated cultures as well as controls were run in triplicate.

Cultures were incubated while shaking at approximately 60 excursions/min on Model G2 Gyratory shakers fitted with No. AG2-Ta25 platforms (New Brunswick Scientific Co., New Brunswick, CN) under 150 µE/m^2/sec PAR illumination from "cool-white" fluorescent tubes; temperature was 20.0 ± 0.5°C; the lighting cycle was 14 h of light followed by 10 h of darkness; test duration was 96 h.

After 96 h incubation, absorbances of cultures and optical blanks were measured at 525 nm on a Fisher Electrophotometer II^{R} (Fisher Scientific Co., Pittsburg, PA).

Concentrations that inhibited growth by 50% (EC50) and 95% confidence intervals were calculated by moving averages on a Digital Equipment Corporation PDP 11/70 computer.

INVERTEBRATE AND FISH TOXICITY TESTS

Animal Species

Invertebrates and fish used in static acute toxicity tests were spawned or cultured at ERL, GB. The parent Eastern oyster (Crassostrea virginica)

stock was purchased from oystermen, then conditioned and induced to spawn at ERL, GB. Gametes were mixed to produce <2-h-old embryos for 48 h EC50 tests.

Stone crab (Menippe mercenaria) zoea larvae were hatched from a gravid female collected from Santa Rosa Sound at Sabine Island. Mysid shrimp (Mysidopsis bahia) <24-h-old were hatched from brooding females from laboratory mysid cultures isolated overnight in a brood chamber.

Sheepshead minnow (Cyprinodon variegatus) brood stock was captured from embayments on Santa Rosa Island, then conditioned and induced to spawn in the laboratory. Fry for toxicity tests were reared to 28 days of age.

Animal Food

Oyster parent stock were fed on natural plankton assemblages in unfiltered seawater, while oyster larvae were not fed. Stone crab adult females were fed raw fish muscle. Mysid shrimp and stone crab larvae were fed living brine shrimp (Artemia sp.) nauplii during culture. The sheepshead minnow brood stock diet included fresh-frozen adult Artemia and a commercial dried flake food. Fry were fed Artemia nauplii.

Chemical analysis of all foods given to brood stock or test animals indicated that non detectable concentrations of chlorinated hydrocarbons less than 0.1 (µg/g) or parts per million (ppm) wet weight were present.

With the exception of adult oysters, which received raw unfiltered seawater, all animals were spawned or cultured in intermittent-flow seawater filtered through coarse sand and 10 to 20 micrometer (µm) polypropylene-core filters at ambient salinity and 25°C. Static toxicity tests were conducted in seawater filtered through 1.0 µm polypropylene-core filters and adjusted to 20 °/oo salinity by addition of Rila Salt Mix® (Rila Products, Teaneck, NJ) or deionized water. Temperature was maintained at 25 ± 1°C in all cultures and toxicity tests.

Test Pesticides

Pesticide samples used in this study are described in Appendix A. Seawater concentrations and median effect concentrations of each pesticide are reported here as micrograms (µg) of pesticide per liter (ℓ) of seawater, or parts per billion. All concentrations are nominal based on weight of sample per volume and are uncorrected for purity or specific gravity.

Static Acute Toxicity Tests

All toxicity tests were conducted in accordance with Draft Toxicity Test Standards of the American Society of Testing and Materials; 48-h EC50s for larval oysters in accordance with "Standard Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs" (ASTM, 1978a), and 96-h LC50s for stone crab larvae, mysids, and fish in accordance with the proposed "New Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" (ASTM, 1978b).

During the 96-h test period, stone crab larvae and mysid shrimp were fed 48-h-old Artemia nauplii each day to minimize cannibalism and starvation.

Both 48-h EC50s (and 95% confidence intervals) for Eastern oyster larvae and 96-h LC50s (and 95% confidence intervals) for crustaceans and fish were calculated by either the probit, binomial, or moving averages.

RESULTS AND DISCUSSION

Results of algal bioassays (Table 1) and static acute toxicity tests with invertebrates and fish (Table 2) provide an initial foundation for the toxicological assessment of Ambush, Bolero, Bux, Dursban, Fentrifanil, Larvin, and Pydrin on estuarine organisms. The 48-h EC50 for Eastern oyster larvae was not determined since this species was determined to be insensitive to four pesticides in a short-term acute exposure. The estimate of toxicity (expressed as 48-h EC50 >1,000 mg/ℓ) provides a confirmatory result. Test concentrations >1,000 mg/ℓ cause cross-contamination of equipment and glassware and increase the exposure hazard to workers. When an estuarine test species is insensitive at 1,000 mg/ℓ, further acute testing should be continued until toxicity to several phyla has been determined.

The synthetic pyrethroids, Ambush and Pydrin, were the most toxic of the seven pesticides tested and were extremely toxic to crustaceans. Toxicity, in decreasing order, to algal population growth (Table 1) was Ambush, Dursban, Bolero, Larvin, Pydrin, and oyster larvae (Table 2), Ambush, Pydrin, Dursban, Bolero. Acute lethality, in decreasing order, to crustaceans was Pydrin, Ambush, Dursban, Fentrifanil, Bux, Larvin, Bolero and to fish, Fentrifanil, Bux, Ambush, Pydrin, Dursban, Larvin, Bolero.

Bolero, the only herbicide tested, was less toxic than the six insecticides. Phylogenetically, the descending order of sensitivity was: crustaceans, fish, algae, and oyster larvae.

The synthetic pyrethroids were extremely insoluble in seawater. In tests with algae, oyster larvae, and fish, median effects were higher than the solubility in seawater. Consequently, actual exposure concentrations of Ambush and Pydrin were probably much lower than the dosed nominal concentrations due to their hydrophobic nature. Results of several pyrethroid toxicity tests with fish varied, indicating that exposure concentrations (above solubility) were inconsistent among tests. A subsequent report from ERL,GB (Schimmel et al., 1981) confirms that pyrethroid toxicities in flowing acute tests with sheepshead minnows were an order of magnitude lower than in static acute tests reported here. Since problems with pyrethroid exposure concentrations occurred with fish, static acute values reported here should not be considered final. Crustaceans were extremely sensitive to Ambush and Pydrin at concentrations below the limit of solubility in seawater. When compared to flowing acute test results, static tests with mysid shrimp were characteristically 2 and 3 X less sensitive.

REFERENCES

American Society for Testing and Materials Committee E-35 on Pesticides, 1978a. Proposed Standard Practice for Conducting Static Basic Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs. Draft No. 4., 34p.

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Schimmel, Steven C., Richard L. Garnas, James M. Patrick, Jr., and James C. Moore. 1981. Acute Toxicity, Bioconcentration, and Persistence of AC 222,705, Benthiocarb, Chlorpyrifos, Fenvalerate, Methyl Parathion, and Permethrin in the Estuarine Environment. (Submitted to Journal of Agriculture and Food Chemistry).

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Table 1. Results of algal bioassays using pesticides and unicellular algae. Values are the 96-h EC50s (95% confidence interval), or the calculated concentration that would inhibit growth by 50%, as compared to control growth, expressed in units of parts per billion (micrograms per liter).

Pesticide	Species Tested	Test No.	96-h EC50 (95%CI) µg/ℓ
Ambush	Skeletonema costatum	1	124 (104-154)
		2	92 (71-115)
Bolero	Skeletonema costatum	1	459 (327-692)
		2	410 (368-461)
		3	327 (290-368)
		4	377 (326-439)
		5	389 (338-452)
Dursban	Skeletonema costatum	1	255 (228-286)
		2	289 (257-334)
		3	326 (283-402)
		4	328 (290-391)
		5	297 (268-335)
	Isochrysis galbana	1	138 (112-169)
	Thalassiosira pseudonana	1	148 (121-182)
Larvin	Skeletonema costatum	1	457 (386-547)
		2	462 (414-518)
		3	404 (366-449)
		4	454 (410-509)
Pydrin	Skeletonema costatum	1	>1,000*
	Isochrysis galbana		>1,000*
	Thalassiosira pseudonana		>1,000*
	Nitzschia angularum		>1,000*

* No effect on growth

Table 2. Acute static toxicity of pesticides to several estaurine animals. Values are 96-h LC50 (95% confidence interval) or *48-h EC50 (based on abnormal development) expressed in units of parts per billion (micrograms/liter).

Pesticide	*Crassostrea virginica <2-h larvae	Mysidopsis bahia 1-d juveniles	Menippe mercenaria zoea larvae	Cyprinodon variegatus 28-d fry
Ambush	>1,000	0.046(0.032-0.056)	0.018(0.010-0.032)	88(82-95)
Bolero	1,000<X<10,000	370(317-433)	-	>1,000
Bux	-	1.0 by graphical interpolation	-	46(41-51)
Dursban	1,991(1,505-2,809)	0.056(0.032-0.100)	-	270(235-309)
Fentrifanil	-	0.01<X<0.10	-	9(8-10)
Larvin	-	263(229-299)	-	>1,000
Pydrin	>1,000	0.021(0.019-0.024)	-	121(96-149)

Note: Greater than (>) values are the result of confirmatory tests.
Ranges (<X<) indicates results of range-finder tests.

Appendix A. Pesticide Sources, Purities, Major Pests, Applications, and Registration Status

Parameter	REGISTERED TRADENAMES						
	Ambush[a]	Bolero[b]	Bux[c]	Dursban[d]	Fentrifanil[e] PP 199	Larvin[f]	Pydrin[g]
Common Name	Permethrin	Thiobencarb	Bufencarb	Chlorpyrifos	Fentrifanil		Fenvalerate
Technical Material Source	ICI Americas, Inc.	Chevron Chemical Co.	Chevron Chemical Co.	Dow Chemical Co.	ICI United States, Inc.	Union Carbide	Shell Development Co.
Lot	47/82	10391	Analytical Standard, from RTP[h]	1-5503-2	Analytical Standard from RTP	511-01-2828	80115
Purity,%							
Manufacturer	93	95.5	--	97.7	--	99	--
In-house	93	90	--	92	--	--	100
Date Received	5/4/79	5/15/79	--	4/24/79	--	6/7/79	4/20/79
Use	Insecticide	Herbicide	Insecticide	Insecticide	Insecticde	Insecticide	Insecticide
Major Target Pests	budworm bollworm bollweevil	preemergent and early postemergent grasses and broadleafs	corn rootworm rice water weevil	fire ants household pests worms scale borers	mites aphids scale insects	lepidopterous pests	budworms bollworm bollweevil
Application	Cotton	Rice	Corn Rice	soil turf foliage cotton corn cattle	apples citrus	cotton soybeans	cotton vegetables fruit
Status	Registered (uses pending)	Section 18 Emergency Exemption	Registered	Registered (uses pending)	Experimental Use Permit	Experimental Use Permit	Registered (uses pending)

[a] 3-(phenoxyphenyl) methyl(±)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate (approx. 60% trans, 40% cis isomers)

[b] S-(4-chlorophenyl) methyl diethylcarbamothioate

[c] mixture of m-(ethylpropyl) phenyl methylcarbamate and m-(1-methylbutyl) phenyl methylcarbamate in approx. ratio of 1:3

[d] O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate

[e] N-[2-chloro-5-(trifluoromethyl) phenyl]-2,4-dinitro-6-(trifluoromethyl) benzenamine

[f] ethanimidothioic acid, N,N[1] -[thiobis[(methyl-imino)carbonyloxy]]bis-dimethylester

[g] cyano (3-phenoxyphenyl) methyl 4-chloro-alpha (1-methylethyl) benzeneacetate

Appendix B

Composition of mixes added to algal growth media

Nutrient Mixes	Amount per liter
Metal Mix[a]	
$FeCl_3$-$6H_2O$	0.048g
$MnCl_2 \cdot 4H_2O$	0.144g
$ZnSO_4 \cdot 7H_2O$	0.045g
$CuSO_4 \cdot 5H_2O$	0.157mg
$CoCl_2 \cdot 6H_2O$	0.404mg
H_3BO_3	1.140g
Na_2EDTA[d]	1g
Glass-distilled or deionized water	1ℓ
Vitamin mix[b]	
Thiamin hydrochloride	50mg
Biotin	0.01mg
B_{12}	0.10mg
Glass-distilled or deionized water	100mℓ
Minor Salt Mix[c]	
K_3PO_4	3.0g
$NaNO_3$	50.0g
$Na_2SiO_3 \cdot 9H_2O$	20.0g
Glass-distilled or deionized water	1ℓ

[a]Add 15.0 mℓ/ℓ of test solution.

[b]Add 0.5 mℓ/ℓ of test solution.

[c]Add 1.0 mℓ/ℓ of test solution.

[d]EDTA added only to stock medium.